Data Evaluation Report on the Acute Toxicity of RPA 407213 to Freshwater Invertebrates - *Daphnia magna*

PMRA Submission Number{.....} EPA MRID Number 45385724

Data Requirement: PMRA DATA CODE: {.....}
EPA DP Barcode: D275213
OECD Data Point:
EPA MRID: 45385724
EPA Guideline: 72-2

Test material: RPA 407213 **Purity:** 99.8% RPA 407213 & 0.2% RPA 407212
Common name: Fenamidone
Chemical name: (+)-(4S)-4-methyl-2-methylthio-4-phenyl-(1H)-1-phenylamino-2-imidazolin-5-one
CAS name: (5S)-3,5-dihydro-5-methyl-2-methylthio-5-phenyl-3-phenylamino-4H-imidazol-4-one
CAS No.: 161326-34-7
Synonyms: Not reported

Primary Reviewer: Mary Thomas, M.S. **Signature:** Mary Thomas
Staff Scientist, Dynamac Corporation **Date:** 2/15/02

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Secondary Reviewer(s): {.....} **Date:** {.....} 10/2/02
{EPA/OECD/PMRA}

Reference/Submission No.: {.....}

Company Code {.....} [For PMRA]
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Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: Suteau, P. 1997. RPA 407213 Acute toxicity (48 hours) to Daphnids *(Daphnia magna)* under semi-static conditions. Unpublished study performed by Rhône-Poulenc Agrochimie, Centre de Recherche, 355, rue Dostoïevski, BP 153, F-06903 Sophia Antipolis Cedex and sponsored by Rhône-Poulenc Agrochimie, 14-20, rue Pierre Baizet, BP 9163, F-69263 Lyon Cedex 09. Study number: SA 96433. Study initiated on October 15, 1996 and completed on January 24, 1997.

EXECUTIVE SUMMARY:

The 48-hr-acute toxicity of RPA 407213 to *Daphnia magna* was studied under semi-static conditions. Dilution water control served as a comparison to five nominal concentrations of RPA 407213 of 0.04, 0.06, 0.10, 0.18 and 0.30 mg/L. Mean measured concentrations of RPA 407213 were 0 (control), 0 (solvent), 0.03, 0.06, 0.11, 0.19 and 0.32 mg a.i./L. The 48-hour EC_{50} was 0.19 mg a.i./L, based on mean measured concentrations. As a result, RPA 407213 is classified as highly toxic to *Daphnia magna* on an acute toxicity basis. The 48-hr- NOAEC based on sublethal effects was 0.06 mg/L. Sublethal effects included immobilization at treatment levels greater than 0.06 mg a.i./L.

This study is scientifically sound, but it only partially satisfies the guideline requirements for an acute toxicity study with freshwater invertebrates. The water hardness and the pH varied substantially from the US EPA guideline recommendations. This study is classified as Supplemental.

Results Synopsis

Test Organism Age (e.g. 1st instar): ≤24 hrs. old
Test Type (Flow-through, Static, Static Renewal): Static Renewal

LC50: N/A	95% C.I.: N/A
24- hour EC50: 0.25 mg a.i./L	95% C.I.: 0.21 to 0.30 mg a.i./L
48- hour EC50: 0.19 mg a.i./L	95% C.I.: 0.16 to 0.22 mg a.i./L
NOAEC (48 hours): 0.06 mg a.i./L	

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: OECD guideline N° 202 I (1984), E.E.C. directive 92/69- method C2 (1992) and E.P.A./FIFRA guideline 72-2 (1985).

Deviations included:

1. Water hardness (170 mg/L $CaCO_3$), was significantly higher than the range that is recommended by US EPA (40-48 mg/L as $CaCO_3$). The pH was also higher (7.8) than recommended (7.2-7.6). Because water hardness and pH are properties that can influence the bioavaliability of the test material to daphnids, these deviations impacted the acceptability, but not the validity of this study.

2. The study author failed to report the OECD test chemical physical characteristics (i.e., water solubility, vapor pressure, and specific activity).

3. Biomass loading rate was not reported.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material RPA 407213

Description: White powder

Lot/Batch No. : MDA9607

Purity: 99.8% RPA 407213 & 0.2% RPA 407212

Stability of Compound Under Test Conditions: Mean measured concentrations of expired solutions (24 and 48 hours) ranged from 91-133% of mean measured concentrations of fresh solutions (0 and 24 hours), showing that the test material was stable under test conditions. OECD requirements were not reported.

Water solubility: Not reported
Vapor pressure: Not reported
Specific activity: Not reported
Molecular weight: 311.41

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of test chemicals: The test material was stored in the dark in an air-tight container, at room temperature (approximately 20°C).

2. Test organism:

Species: *Daphnia magna* *EPA preferred species is Daphnia magna*
Age at test initiation: <24 hrs. old
Source: Clone 5 originating from INERIS Laboratory (BP1-91710 Vert-le-petit, France).

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: Nominal concentrations were reportedly based on results from previous toxicity tests with daphnids. A range finding study was not reported.

b) Definitive Study

Table 1. Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	N/A Same as test. *Daphnia* cultures were fed a combination of flake fish food (Tetramin), nutrient broth, yeast suspension, seaweed extract (Marinure 30) and unicellular green algae (*Chlorella vulgaris*) three times weekly. During the course of the study *Daphnia* were not fed. Prior to the test period *Daphnia* were healthy.	Dilution water was same as culture water *EPA requires 7 day minimum acclimation period* *No feeding during study*
Duration of the test	48 hours	*EPA requires 48 hours*
Test condition static/flow through Type of dilution system- for flow through method. Renewal rate for static renewal	Static renewal test N/A 24 hours	*EPA requires consistent flow rate of 5 - 10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	Prior to the study period, the culture medium was continuously aerated (air bubbling). The test solutions were not aerated.	
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	 Glass 250 mL 200 mL	 *EPA requires: size 250 mL or 3.9 L fill 200 mL*

Parameter	Details	Remarks *Criteria*
Source of dilution water	Reconstituted water 80% DSW + 20% LC-oligo; see Appendix 2, pp. 45-46).	*EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water.*
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 170 mg/L $CaCO_3$ 7.8 ≥8.5 mg/L 19.4°C-20.6°C Not reported Not reported p. 33 p. 34 Not reported	Water hardness was significantly higher than required by EPA. The pH was also higher than recommended. *EPA requires:* *hardness: 40 - 48 mg/L as $CaCO_3$* *pH: 7.2 - 7.6* *-Temperature: 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > 1°C* *Dissolved oxygen:* *Static: ≥ 60% during 1st 48 hr and ≥ 40% during 2nd 48 hr* *Flow-through: ≥60%*
Number of replicates Solvent control: Negative control: Treatments:	 1 1 2	
Number of organisms per replicate Solvent control: Negative control: Treatments:	 1 1 10 per replicate; 2 replicates per treatment level	Five treatment levels plus water control with 20 *Daphnia* per treatment. *EPA requires 5 treatment levels plus control with a minimum of 20 daphnid per treatment. Biomass loading rate for static ≤ 0.8 g.L at ≤ 17oC, ≤ 0.5 g/L at > 17oC; flow-through: ≤ 1 g/L/day.*

Parameter	Details	Remarks *Criteria*
Treatment concentrations nominal: measured:	Water control, solvent control, 0.04, 0.06, 0.10, 0.18 and 0.30 mg a.i./L RPA 407213 <0.02,<0.02, 0.03, 0.06, 0.11, 0.19 and 0.32 mg a.i./L RPA 407213	Mean measured concentrations are the average of samples analyzed on days 0, and 2. *EPA requires a geometric series with each concentration being at least 60% of the next higher one.*
Solvent (type, percentage, if used)	Dimethyl formamide 0.1 mL/L	*EPA requires solvents not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-though tests.*
Lighting	16 hours light and 8 hours dark	*EPA requires 16 hours light, 8 hours dark.*
Stability of chemical in the test system	The test material was stable under test conditions. Mean measured concentrations of expired solutions (24 and 48 hours) ranged from 91-133% of mean measured concentrations of fresh solutions (0 and 24 hours).	
Recovery of chemical Level of Quantitation Level of Detection	100-133% 0.020 mg/L Not reported	
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured including the sublethal effects	Sublethal effects (immobilization)	
Observation intervals	3, 24, and 48 hours	
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. SUB-LETHAL TOXICITY ENDPOINTS:

Immobilization was observed at all treatment levels by 24 and 48 hours. In the 0.11, 0.19 and 0.32 treatment levels, 5, 50, and 95% of daphnids were immobilized at 48 hours, respectively.

Table 3: Effect of RPA 407213 on the immobilization of *Daphnia magna.*

Treatment (mg a.i./L) Mean Measured and (Nominal)	Observation period			
	Day 24		Day 48	
	endpoint	% affected	endpoint	% affected
Dilution water control	Immobilization	0	Immobilization	0
Solvent control	Immobilization	0	Immobilization	0
Positive control, if used	N/A	N/A	N/A	N/A
0.03 (0.04)	Immobilization	0	Immobilization	0
0.06 (0.06)	Immobilization	0	Immobilization	0
0.11 (0.10)	Immobilization	5	Immobilization	5
0.19 (0.18)	Immobilization	5	Immobilization	50
0.32 (0.30)	Immobilization	10	Immobilization	95
NOAEC mg/L	--	NR	--	0.11
LOAEC	--	NR	--	0.19

Treatment (mg a.i./L) Mean Measured and (Nominal)	Observation period			
	Day 24		Day 48	
	endpoint	% affected	endpoint	% affected
EC_{50} mg a.i./L	--	0.25	--	0.19mg/L

NR = Not reported.

C. REPORTED STATISTICS:

The study author indicated that probit method was used to calculate the EC50 value and its 95% confidence interval.

LC50: N/A 95% C.I.: N/A
24- hour EC50: 0.25 mg a.i./L 95% C.I.: 0.21 to 0.30 mg a.i./L
48- hour EC50: 0.19 mg a.i./L 95% C.I.: 0.16 to 0.22 mg a.i./L
NOAEC (48 hours): 0.06 mg a.i./L

D. VERIFICATION OF STATISTICAL RESULTS:

The 24 and 48 hour EC_{50} values and confidence intervals were estimated using the probit method via ToxAnal .software. The NOAEC was determined by comparing the control to the treatment means using Fisher's Exact test.

LC50: N/A 95% C.I.: N/A
24- hour EC50: 0.25 mg a.i./L 95% C.I.: 0.21 to 0.30 mg a.i./L
48- hour EC50: 0.19 mg a.i./L 95% C.I.: 0.16 to 0.22 mg a.i./L
NOAEC (48 hours): 0.06 mg a.i./L

E. STUDY DEFICIENCIES:

The water hardness in this study was substantially higher (170 mg/L $CaCO_3$) than that recommended by US EPA (40-48 mg/L $CaCO_3$), and the pH of the test solution also varied outside the range recommended by US EPA. Because these parameters may influence the bioavailability of toxins to daphnids, these deviations impacted the acceptability of this study.

F. REVIEWER'S COMMENTS:

The reviewer's statistical results were identical to those reported by the study author.

G. CONCLUSIONS:

This study is scientifically sound, but it only partially fulfills EPA guidelines for toxicity testing with freshwater invertebrates (§72-2). This study is classified as Supplemental because water hardness and pH varied substantially from US EPA recommendations. The 48-hour EC_{50} was determined to be 0.19 mg/L which classifies RPA 407213 as highly toxic to daphnids on an acute toxicity basis. The NOAEC was determined to be 0.06 mg/L, based on immobilization.

LC50: N/A 95% C.I.: N/A
24- hour EC50: 0.25 mg a.i./L 95% C.I.: 0.21 to 0.30 mg a.i./L
48- hour EC50: 0.19 mg a.i./L 95% C.I.: 0.16 to 0.22 mg a.i./L
NOAEC (48 hours): 0.06 mg a.i./L

III. REFERENCES:

1) ASTM (1994) - Standard guide for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. E729-88a, American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA, 19103 - 1187 USA.

2) E.E.C. (1992) - Annex to Commission Directive 92/69/E.E.C. of 31/07/92 - Part C, Methods of determination of Ecotoxicity - Method C2: Acute toxicity to daphnids, Official Journal of European Communities, Publication n° L 383 A, pp 172-178.

3) US E.P.A. (1985): Hazard Evaluation Division - Standard Evaluation Procedure - Acute toxicity test for freshwater invertebrates (EPA540/9-85-005), FIFRA guideline n°72-2.

4) OECD (1984) - Guidelines for testing of Chemicals - Section 2: Effects on biotic systems: 202 - *Daphnia sp.*, Acute immobilisation test and reproduction test - Part I - The 24-hour EC_{50} acute immobilization test.

5) RPA 407213: Analytical determination method in freshwater for ecotoxicology: ANL/055-94E. G. Lafay, J. P. Oullier, J. P. Tassel, M. Vincent, A. Soun, Rhône-Poulenc Agrochimie, Sophia Antipolis Research Center, 1994.

6) Stephan C E (1977). Methods for calculating an LC50 in: Aquatic Toxicology and Hazard Evaluation, ASTM STP 634, Mayer, F. L and Hamelink J. L. (eds), pp 65-84.